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Meningeal lymphatic vessels in the human head: Examples of *in vivo* visualization with high-resolution 3T MRI

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Abstract

In 2015, meningeal lymphatic vessels (mLVs) were (re)discovered in mice and human dura specimens. Two years later, the first report was published showing that mLVs can be detected in humans *in vivo* by high-resolution 3 Tesla magnetic resonance imaging (MRI). In 2017 and 2018, two further studies reported the successful MRI-based detection of mLVs *in vivo* in humans. The aim of our study was to provide further evidence of the possibility to detect mLVs *in vivo* with MRI in humans. To this end, MR images already available from one subject (the first author) were analyzed. We detected mLVs in the coronal plane at the bilateral superior lateral corners of the superior sagittal sinus (SSS) as well as below the SSS, in agreement with the two other published reports about the *in vivo* detection of mLVS in humans with MRI. Our report is thus, to the best of our knowledge, the fourth published report about *in vivo* MR imaging of human mLVs.

Introduction

In 2015, Aspelund et al. [1] and Louveau et al. [2] reported the (re)discovery of meningeal lymphatic vessels (mLVs) in mice. Previous century-old reports (going back till the end of the 18th century) about lymphatic vessels in the cranium were wrongly disputed and dismissed in the past [3]. The cranial lymphatic system (CLS) of mice was described by Aspelund et al. and Louveau et al. as lying next to the cerebral arterial and venous system, i.e. running down to the base of the skull along the superior sagittal sinus (SSS), transverse sinus, straight sinus, sigmoid sinus, the retroglenoid vein and branches of the middle and anterior meningeal arteries. In 2015, Louveau et al. [2] reported also the first indication of mLVs being present at the SSS in humans. To this end, an *in vitro* analysis of autopsy specimens of the human dura was performed.

In 2017, Absinta et al. [4] published the first report showing that mLVs can be detected in humans *in vivo* by high-resolution 3 Tesla magnetic resonance imaging (MRI), proving that a CLS exist also in humans. In 2018, Kuo et al. [5] also showed that 3T MRI is able to image the mLVs *in vivo* in humans. In 2019, Naganawa et al. [6] confirmed the findings of Absinta et al. [4] and Kuo et al. [5] by showing that MR imaging with a 3D-real inversion recovery (3D-real IR) sequence is able to detect MLVs *in vivo*. At present, there are, therefore, to the best of our knowledge, only three published research reports about the *in vivo* MRI-based detection of mLVs in humans.

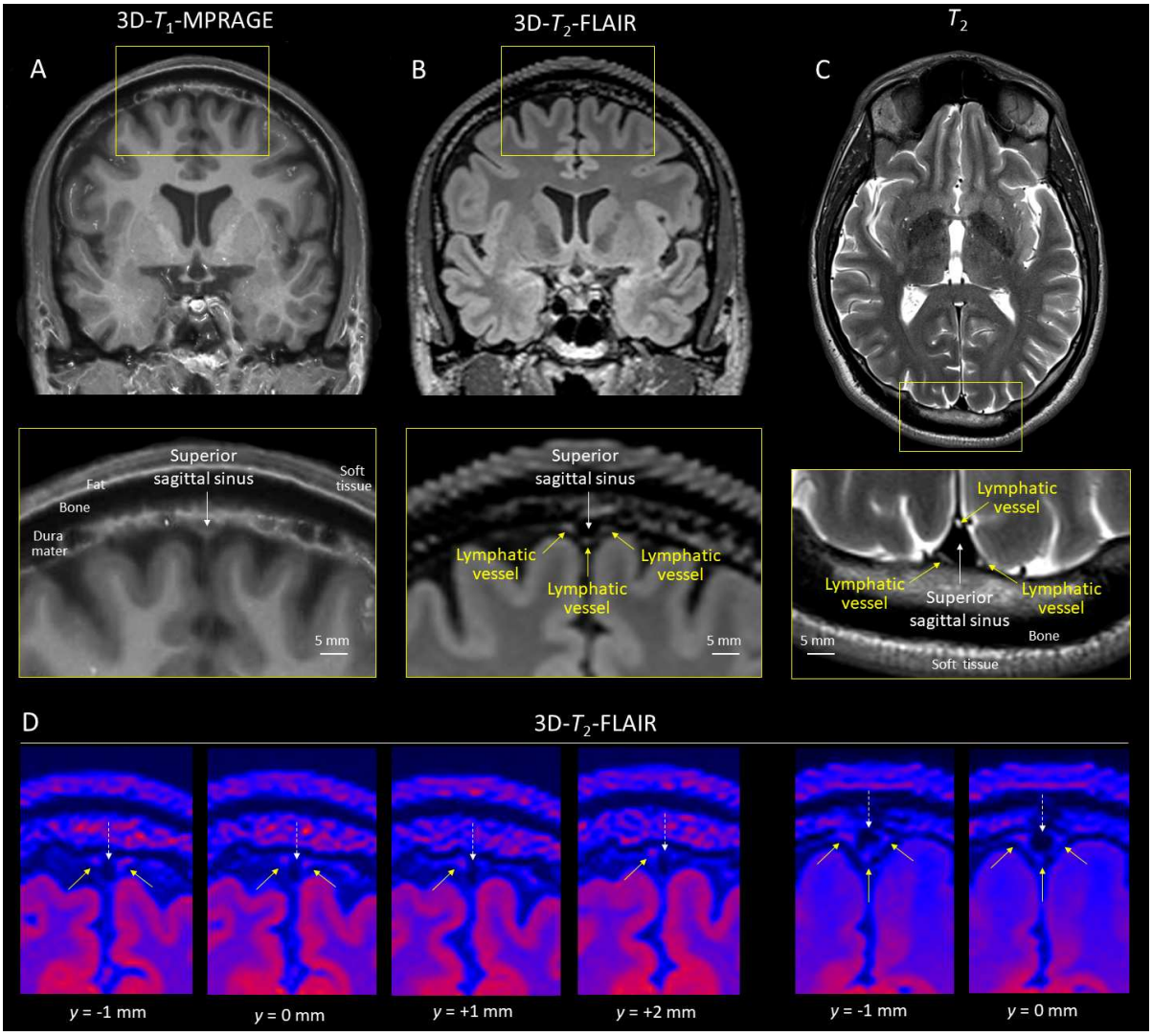
The last couple of years witnessed an increasing interest in and understanding about the CLS both from an anatomical, functional and pathophysiological point of view (for a review see [7] [8] [9] [10] [11] [12] [13] [14] [15]). For example, Kuo et al. [5] discovered that the lymph flow in the mLVs in the SSS in humans runs countercurrent to the venous blood flow. Glinskii et al. [16] recently showed in mice that there seem to be also non-sinus-associated lymphatic structures throughout the dura mater as well as microvascular structures with a dual blood vasculature and lymphatic endothelial identity, highlighting the complexity of morphology and structure of the CLS. Goodman et al. [17] demonstrated with an *in vitro* analysis of specimens of human dura that two types of mLVs next to the SSS can be distinguished based on their morphology and localization: mLVs of type 1 (morphology: single layer of endothelium, devoid of smooth muscle or red blood cells, unoccupied lumen, irregular morphology; LYVE-1 (lymphatic vessel endothelial hyaluronan receptor 1) positive; location: predominately within the

periosteal and meningeal layer of the dura mater) and mLVs of type 2 (morphology: irregular endothelial border, occupied lumen; LYVE-1 negative; location: predominately between the SSS and periosteal layer of the dura mater). Recently, Ahn et al. [18] showed that mLVs from the dorsal part of the skull (located within the dura at the SSS and transverse sinus) seem to be functionally different to mLVs from the basal part of the skull: basal mLVs were found to be hotspots for lymphatic drainage of cerebrospinal fluid (CSF). Interestingly, the paper of Ahn et al. [18] and well as the work published by Ma et al. [19] were not able to reproduce the findings of the group of Kipnis about the CSF drainage into the dorsal network of mLVs. It is currently not absolutely clear if the mLVs drain CSR or if CSF rather drains along cranial and spinal nerves to research lymphatic vessels outside the skull (for a discussion see [20]). The work of Ma et al. [19] showed results in favor of the second option.

The exploration of the CLS is increasingly attracting research effort to further understand its anatomical and functional role in health and disease.

Objective

Our objective was to replicate the findings of Absinta et al. [4] who provided the first *in vivo* evidence of the existence of mLVs in humans. To this end, MRI images of the human head from the first author were obtained with the same type of high-resolution MRI machine (Skyra, Siemens Healthcare) as used by Absinta et al. and a detailed analysis of the images was performed.



a

Figure Legend

Figure 1. Visualization of meningeal lymphatic vessels imaged with MRI.

(A) Coronal 3D-T_1-MPRAGE image. The superior sagittal sinus, SSS, is clearly visible as a bright triangular area (white arrow). Meningeal lymphatic vessels are not visible. The coronal slices were created based on transaxial images; tiny white dots and light-reflection like features are artifacts due to the 3D rendering and visualization performed to obtain the coronal image view.

(B) Coronal 3D-T_2-FLAIR image of the same coronal plane as in **(A)**. While the SSS is visible as a dark triangular area (white arrow), the meningeal lymphatic vessels left, right and below the SSS are visible as bright spots.

(C) Transverse 2D-T_2 image. As in **(B)**, SSS is visible as a dark triangular area (white arrow), the meningeal lymphatic vessels left, right and bellow the SSS are visible as bright spots.

(D) Coronal 3D-T_2-FLAIR image sequence in false-colors to highlight the spatial presence of the meningeal lymphatic vessels at two regions of interest (4 mm stack: 4 images at left; 2 mm stack: 2 images at right). The SSS is marked by a dotted white arrow, the meningeal lymphatic vessels by yellow arrows. The images are shown in false colors to highlights details and to increase the contrast.

Results & Discussion

3 mLVs were found in the coronal T_2-weighted 3-dimensional fluid-attenuated inversion recovery sequence (3D-T_2-FLAIR) image of the same coronal plane as shown in figure 1 of Absinta et al. [4], 2 mLVs could be identified in the coronal plane at the bilateral superior lateral corners of the SSS as well as below the SSS (Fig. 1B). The mLVs were visible in multiple coronal sections at different regions of interest at the dorsal side of the brain surface. Figure 1D shows 2 image sequences where the course of the mLVs is visible.

Coronal T_1-weighted 3-dimensional magnetization-prepared rapid gradient-echo sequence (3D-T_1- MPRAGE) image of the same section shows no visible mLVs (as expected) but the SSS as a triangular area filled with an intermediate intensity (as usually observed) (Fig. 1A).

Transverse 2D-T_2-weighted images showed clearly high-intensity regions at the occipital part of the brain surface most likely to also resemble mLVs (Fig. 1C).

The diameter of the mLVs was determined from successive 3D-T_2-FLAIR images of the superior region shown in figure 1B to be 700 ± 300 µm and at the occipital region based on the 2D-T_2 images to be 800 ± 300 µm. This is in general agreement with the results of Goodman et al. [17] who determined the diameter of mLVs from extracted human meningeal samples to be 354 ± 55 µm (healthy adults) and 381 ± 76 (subjects with Alzheimer's disease) within a range of 19 to 470 µm.

With our study, we could replicate the findings of Absinta et al. [4] and Kuo et al. [5]. In particular, we showed that mLVs are visible in high-resolution 3D-T_2-FLAIR and 2D-T_2 MR images. To the best of our knowledge, our report is the fourth published report (with Absinta et al. [4] being the first, Kuo et al. [5] being the second and Naganawa et al. [6] being the third) demonstrating the successful *in vivo* MRI-based detection of mLVs in humans. Since no gadolinium-based contrast agents (GBCA) were intravenously administered for MR imaging in our study – in contrast to the study of Absinta et al. – our findings also highlight the fact that mLVs can be observed in T_2-weighted MR images even without GBCA contrast enhancement. This finding is also in agreement with the results of Kuo et al. [5] where also no GBCA was used.

Our findings of the position of the mLVs in the dura as well as the number of mLVs around the SSS are aligned with the findings of Absinta et al. [4]. Furthermore, the diameter of the mLVs estimated by us based on the MRI images is in good agreement with the results of Absinta et al. [4]. Our findings also support those of Naganawa et al. [6] who used a 3D-real IR sequence; however, Naganawa et al. did not report finding mLVs below the SSS as we and Absinta et al. did. A mLV below the SSS was also detected by Kuo et al. [5], strengthening our findings that indeed 3 mLVs surround the SSS. Interestingly, in the *ex vivo* analysis of specimens of human dura conducted by Goodman et al. [17] more than 5 vessels were found around the SSS.

We found mLVs in the occipital part of the head in 2D-T_2 images. The presence of mLVs occipitally in humans is also in agreement with the findings of Absinta et al. that showed the presence of putative mLVs at the same position using 3D rendering (see Suppl. Fig. 1 of Absinta et al. [4]).

We think it is unlikely that the mLVs identified by us are actually arachnoid granulations since the structures found had a tubular form where arachnoid granulations do not have this feature.

Conclusions

Our report demonstrated the possibility to detect mLVs *in vivo* based on MR imaging and should encourage all researchers and medical professionals to analyze MR images from the human head to routinely look for mVLs in these images in order to include also the assessment of mLVs when analyzing MR images.

Neurosurgeons that (intentionally or unintentionally) perform a durotomy should be aware that disruption of mLVs might have pathophysiological consequences.

We are sure that the investigation of the CLS will continue to be an important topic in human anatomy and physiology, and the role of the CLS in the pathophysiology of the neurological disease will increasingly emerge in the near future.

To the best of our knowledge, no study was published yet reporting the MRI-based *in vivo* detection of mLVs using ultra-high field magnetic resonance imaging with 7 or 8 T. The increased resolution provided by these devices would make it easier to detect the CLS and would also most probably enable the morphological ultrastructure and filling state of mLVs to be accessed *in vivo*. This could be relevant not only for anatomical and physiological basic research but also in clinical medicine (e.g. diagnosis and disease-course assessment).

Limitations

There are two main limitations of this study. First, the findings reported referring to results from only one subject that underwent MR imaging of the head. Performing MR imaging of more subjects and evaluating replication of the findings with more subjects is of course necessary. However, the fact that one single MR image data set is already sufficient to demonstrate the presence of mLVs in multiple single MRI images is already promising. It demonstrates the relative ease with which mLVs can be detected in MR images when images are available with sufficient resolution and obtained by the 2D-T_2 and 3D-T_2-FLAIR mode. Second, MR imaging in our study was conducted without a contrast-enhancing agent (i.e. without GBCA) at the request of the subject due to the accumulating evidence of the toxicity of GBCAs and the continued debate about this issue [21] [22] [23] [24]). In the study of Absinta et al. [4], a GBCA was used, facilitating easier identification of mLVs in the T_2-weighted images. The fact, that we could identify mLVs also without a GBCA highlights the good image quality of our MR data. It is the second report showing the presence of mLVs *in vivo* in MR images without GBCA administration.

Alternative Explanations

Additional Information

Methods

The subject imaged by MRI was the first author of this report (male, 37 years old, Caucasian). Images were obtained as part of a medical neurological examination at our institute. Imaging of the head was performed with a high-resolution 3 Tesla MRI scanner (Skyra, Siemens Healthcare, Erlangen, Germany) without an intravenously administered GBCA. For the present analysis, MR images obtained with the following sequences were used: 2D-T_2-weighted (repetition time [TR]: 8180 ms, echo time [TE]: 100 ms, slice thickness: 3 mm, the spacing between slices: 3.3 mm, slices: 44, flip angle: 135°, specific absorption rate [SAR]: 0.57 W/kg), T_2-weighted 3-dimensional fluid-attenuated inversion recovery sequence (3D-T_2-FLAIR, TR: 4700 ms, TE: 380 ms, TI: 1800 ms, slice thickness: 1 mm, slices: 250, flip angle: 120°, SAR: 0.2 W/kg) and a T_1-weighted 3-dimensional magnetization-prepared rapid gradient-echo sequence (3D-T_1-MPRAGE, TR: 1790 ms, TE: 2.54 ms, TI: 904 ms, slice thickness: 1 mm, slices: 254, flip angle: 9°, SAR: 0.09 W/kg).

Image analysis and visualization have been performed with iQ-VIEW (IMAGE Information Systems Ltd., Rostock, Germany) and Vesalius 3D (PS-Medtech, Amsterdam, the Netherlands).

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Ethics Statement

The authors declare no conflicts of interest.

MRI images were obtained for medical purposes as part of a neurological diagnostic investigation of the subject. As the subject examined by MRI and the author of this paper is the same, no approval by an ethics committee was necessary. Furthermore, the author has approval by the ethics committee of the Canton Zurich that self-measurements of physiological signals by the authors are allowed and are outside the scope necessary for approval (BASEC-Nr. Req-2018-00951).

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